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ANTIBODIES TO GLUCOSIDES, WITH ESPECIAL REFERENCE TO *RHUS TOXICODENDRON*.*

WILLIAM W. FORD, M D, D P.H.

(From the Bacteriological Laboratory, Johns Hopkins University.)

SOME of the observations which have been made upon poisonous fungi in the laboratory during the past three years have considerable bearing upon general problems in immunity since the conclusions drawn are in direct contradiction to accepted theories upon this subject. It has been shown,[1] for instance, that the aqueous extract of *Amanita phalloides* contains a powerful hemolysin acting upon a variety of blood corpuscles, and in addition a heat-resistant non-hemolytic toxic substance killing animals acutely and producing fatty degenerations. With this aqueous extract it is possible to immunize animals with the production of a serum which has marked anti-hemolytic and low but nevertheless definite antitoxic properties. The antihemolysin is present in a dilution of 1–1,000 of the serum, and 1 c.c. of this serum will neutralize five to eight times a fatal dose of the extract for rabbits or guinea-pigs. It has since been shown in association with Dr. Abel,[2] that the hemolysin is a *glucoside* extremely sensitive to the action of heat, acids, and the digestive ferments, and

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[1] Ford, *Jour. Infect. Dis.*, 1906, 3, p. 191.

[2] *Jour. Biol. Chem.*, 1907, 2, p. 1.

not a "*toxalbumin*" as supposed by Kobert.[1] All proteid may be removed from this glucoside by metaphosphoric acid and by uranyl acetate, leaving its hemolytic activity unaltered. Now if it be possible to immunize animals to this hemolytic substance, producing an antihemolysin, and if, at the same time, it can be shown that this hemolysin is a glucoside, we have consequently produced an *antibody to a glucoside* the possibility of which has been denied by Ehrlich,[2] Bashford,[3] and a number of others. The contentions of the abovementioned observers rest upon a large series of attempts to immunize animals to the glucosides saponin and solanin, and to certain of the poisonous glucosides found in digitalis and in ergot. It had been claimed by one or two observers, notably by Pohl,[4] that after the introduction of small doses of solanin subcutaneously in rabbits, the serum of these animals shows an anti-hemolytic action more marked than does the normal rabbit's serum; and it is still believed by Metchnikoff[5] that the serum of animals treated by non-proteid substances like morphine and arsenic has an increased capacity of neutralizing or oxidizing these substances. In general, however, it may be said that antitoxins have thus far been produced only for bodies supposed to be proteid or proteid derivatives.

These observations upon *Amanita phalloides* thus reopen the entire question, since they must either rest upon fundamental errors in laboratory investigation, or the thesis that antibodies cannot be produced for glucosides does not have a universal application. During the past few months, therefore, we have been studying the action of several of these toxic compounds upon animals, and with one of them the results have been sufficiently constant to merit publication. The substance to which especial attention has been paid is the poisonous glucoside found in *Rhus toxicodendron*, or poison ivy. The active principle of this plant was first properly studied by Pfaff,[6] who found that the irritating poisonous properties reside in the alcoholic extract of the leaves and stem, and the poisonous body was thought by Pfaff

[1] *St. Petersburger med. Wchnschr.*, 1891, 16, pp. 463, 471.

[2] *Chemical Constitution and Pharmacological Action: Collected Studies on Immunity*, New York, 1906, p. 433.

[3] *Arch. internat. de. Pharm. et de Ther.*, 1901, 8, p. 101; 9, p. 451.

[4] *Arch. internat. de Pharm. et de Ther.*, 1900, 7, p. 1; 1901, 8, p. 437.

[5] *Immunity*, Cambridge, 1900.

[6] *Jour. Exper. Med.*, 1897, 2, p. 181.

to be of the nature of a non-volatile oil. It has recently been shown by Syme,[1] working under Acree in Remsen's laboratory, that the active principle of *Rhus toxicodendron* is in reality a *glucoside*, a compound of rhamnose, gallic acid, and fisetin. It is precipitable by lead acetate and upon hydrolysis yields the above substances. The toxic action of the various fractions was tested by Syme upon his own skin, and in this way he was able to follow the poison through the various chemical procedures necessary for its isolation.

Certain clinical phenomena are of interest in connection with poisoning by *Rhus toxicodendron*, and there are vague suggestions in the experience of a number of individuals pointing to an acquisition of artificial immunity. With many persons a small amount of the juice from the fresh leaves is capable of setting up an extensive inflammation of the skin, while others seem able to handle the plant with impunity. At the same time individuals who have been severely poisoned by poison ivy, after a certain number of attacks become accustomed to it, to such an extent at least as no longer to suffer the same serious consequences as at first. Again, certain observers claim to have been cured of an attack and to have been rendered insusceptible to further attacks by the administration of the fluid extract of *Rhus toxicodendron* internally, or by chewing the fresh leaves of the plant. Finally Syme, in his experiments upon himself, found that after four to five months he was no longer susceptible to the poison, although originally his skin had been extremely sensitive. All of these observations are valuable, and while by no means conclusive in showing that immunity may be acquired to this poison, they certainly point out a line for further investigation.

I was enabled to obtain the active principle of *Rhus toxicodendron* in the alcoholic fluid extract of the native plant as prepared by Parke, Davis & Co. This fluid extract contains the poison in workable and practically constant proportions. It had already been shown by Pfaff that the internal administration of his non-volatile oil produced definite lesions in rabbits, the animals dying of an acute nephritis at the end of 14 to 15 days. Occasionally the rabbits died in acute convulsions without any macroscopic brain lesions. The subcutaneous administration of the fluid extract of *Rhus toxicodendron*

[1] *Some Constituents of the Poison Ivy Plant* (*Rhus intoxicodendron*), Johns Hopkins Thesis, 1906.

produces the same effects upon rabbits as those described by Pfaff. Rarely, the rabbits die in convulsions within 24 to 48 hours, but the majority of inoculated animals succumb in from 8 to 15 days. In addition to the nephritis an extensive necrosis and slough is found at the point where the poison is introduced beneath the skin. Following the inoculation we have a fairly long latent period during which the weight of the animals remains stationary. After seven or eight days in a typical case, the animal loses weight rapidly, the necrosis and slough develop, and the animal dies of the nephritis after the lapse of about two weeks. At times the skin lesions are less marked, the damage to the kidney being the important change; an intraperitoneal inoculation seems able to produce these kidney changes with more rapidity than does the subcutaneous method. In addition to rabbits we have found that guinea-pigs are susceptible to the drug, the lesions being produced with greater certainty and regularity. With these animals the necrosis and slough at the point of inoculation are more extensive, while the animals die of the kidney changes in about the same time. The fatal dose of the poison can be estimated for both animals with tolerable accuracy. For guinea-pigs of 250 gram weight, 0.25 c.c. of the alcoholic extract always represent a fatal dose; and a guinea-pig of 350 gram weight practically never survives a dosage of 0.5 c.c. With larger guinea-pigs the proportion of poison to body weight is retained (Table 1).

TABLE 1.

TOXICITY OF *Rhus Toxicodendron* (ALCOHOLIC EXTRACT).

Guinea-Pigs.

Grams Weight	Dosage	Result
	c.c.	
250	1	Death, 13 days
370	0.5	Death, 7 days
265	0.25	Death, 3 days
320	0.20	Recovery
290	0.1	Recovery
380	1	Death, 13 days
350	0.5	Death, 9 days
325	0.25	Death, 10 days

The fatal dose for guinea-pigs can be estimated with considerable accuracy. It may be considered 0.25 c.c. for animals of 250 gram weight; 0.5 c.c. for animals of 350 gram weight.

The fatal dose for young rabbits weighing less than 800 grams can

also be estimated fairly accurately. The animals may die from 0.25 or from 0.5 c.c., but since it is rarely the case that rabbits of this weight may survive this quantity our constantly fatal dose must be placed somewhat higher—at 1 c.c. for a rabbit of 800 grams (Table 2).

TABLE 2.

TOXICITY OF *Rhus Toxicodendron* (ALCOHOLIC EXTRACT).

Rabbits.

Grams Weight	Dosage	Result
	c.c.	
800.....	1	Death, convulsions, 24 hrs.
800.....	1	Death, 10 days
1200.....	1	Death, 9 days
920.....	0.5	Death, 5 days
855.....	0.25	Death, 5 days
850.....	0.25	Recovery
1550.....	1.5	Recovery
1120.....	0.2	Recovery
920.....	0.1	Recovery
1085.....	1	Recovery
1070.....	0.5	Recovery
1330.....	0.75	Recovery

The fatal dose for rabbits of 800 grams is 1 c.c. Certain rabbits of this weight die from smaller doses, but not regularly, and larger animals show greater resistance. Animals of 1,800 to 2,000 gram weight occasionally survive 2 to 3 c.c. doses, but not more than this amount.

Having established the limits of the fatal dosage for both guinea-pigs and rabbits, one or two preliminary experiments were made to determine whether animals which had withstood small doses of the poison were susceptible to amounts of the poison capable of killing untreated animals. For this purpose two small guinea-pigs weighing about 250 grams, which had received 0.2 c.c. and 0.1 c.c. of the *Rhus toxicodendron* extract, were subsequently given 0.5 c.c. and 0.75 c.c. of the poison. One of these animals died in about eight days after the second dose of 0.5 c.c., no immunity being established. The other animal which had received the lower initial dose of 0.1 c.c. reacted well to the second dose of 0.5 c.c. and again to a third dose of 0.75 c.c. representing two to three times a fatal dose. Its condition remained perfectly good, no necrosis or slough appeared at the site of any of the inoculations, and after a period of two weeks the weight which had remained stationary gradually increased to a point above the original weight and remained at this high point during the time

of observation (about three months). At the same time a rabbit weighing 1,550 grams, which after an inoculation of 1.5 c.c. had developed a huge slough at the site of inoculation and a loss in weight from 1,550 to 1,125 grams, but which had later recovered, was given, seven weeks after its original dose, another inoculation of 2 c.c. The second inoculation, larger than the first, with the body weight considerably diminished, produced no slough at the point of injection, and for three weeks the animal remained in good condition. It died then of an infection, and at the autopsy there was no necrosis at the site of inoculation and no evidence of nephritis.

These two animals were suggestive, therefore, in indicating the acquisition of resistance after non-fatal doses, and in giving us certain data for the more serious attempt to establish a high degree of immunity.

The experiments upon this problem were undertaken with four guinea-pigs varying in weight from 450 to 900 grams, and upon nine rabbits varying in weight from 800 to 2,800 grams. In some cases rabbits which had recovered from large initial doses, in testing the limits of the fatal dose, were deliberately given large amounts to test their resistance, while in other instances fresh animals in good condition were employed and the immunization conducted in a more conservative manner. Of these nine rabbits one large Belgian hare died of infection after a final administration of 6 c.c. of the fluid extract. Two rabbits died during the treatment, both after a dose of 6 c.c. With six rabbits in which the amount of the poison given was gradually increased a definite immunity was established.

In these cases the initial doses were small and frequently repeated, and at first a slight loss in weight was noticeable. When the original body weight was regained the immunization was pushed and larger doses were given. The large doses were not given except at fairly infrequent intervals and only after the loss in weight had been made up. With these precautions we were able gradually to give very large amounts of the *Rhus toxicodendron*, amounts so large in all cases as to do away with the factor of high natural resistance on the part of any of the animals.

Thus two of the rabbits had an initial weight of less than 1,000 grams (920 and 850 grams), and in these animals we were able eventu-

ally to give 6 and 7 c.c., representing at least five or six times a fatal dose for rabbits of their weight (Table 3). No subcutaneous lesions developed after even these large doses; the weight of the animals gradually but surely increased, and after they were killed for the collection of their blood serum, there was no evidence of nephritis, the urine being quite free from albumen. These animals received altogether no less than 30 c.c. of the *Rhus toxicodendron* extract, over a period of four months.

TABLE 3.

IMMUNIZATION OF RABBIT (RABBIT 5, WEIGHT 850 GRAMS).

Date	Dosage (*Rhus tox.*)	Grams Weight
	c.c.	
November 5	0.25	850
December 10	0.50	800
" 12		990
" 19	1.0	935
" 21		1000
" 26	1.3	990
" 27		1030
January 4		1095
" 7	1.6	
" 8	No lesion site of inoculation	1140
" 16	2.0	1220
" 25	2.6	1280
February 2	3.25	1300
" 11	4.00	1320
" 21	5.50	1340
" 22		1420
" 25		1400
March 6	7.00	1380
" 16	Killed	1370

An animal with an initial weight of 1,120 grams was eventually given a dose of 8.5 c.c. and killed 10 days after the last inoculation (Table 4). In this case there was a slight amount of albumen in the

TABLE 4.

IMMUNIZATION OF RABBIT (RABBIT 4, WEIGHT 1,120 GRAMS).

Date	Dosage (*Rhus tox.*)	Grams Weight
	c.c.	
November 15	0.2	1,120
December 11	0.8	1,425
" 19	1.2	1,545
" 26	1.6	1,500
" 27		1,635
January 4		1,600
" 7	2.0	
" 8	No lesion at site of inoculation	1,580
" 9		1,635
" 16	2.50	1,695
" 25	3.00	1,835
February 2	4.0	1,830
" 11	5.50	1,780
" 22	7.50	1,790
March 6	8.50	1,930
	Killed March 16; weight 1,890	

urine, but no necrosis at the site of inoculation. Finally three other rabbits weighing originally 1,250, 1,550, and 2,700 grams were pushed to the point where 10 c.c. of *Rhus toxicodendron* was given (Table 5).

TABLE 5.

IMMUNIZATION OF RABBIT (BELGIAN HARE, NO 2; WEIGHT 2,700 GRAMS).

Date	Dosage (*Rhus tox.*)	Grams Weight
	c.c.	
December 4	0.1	2,700
" 6	0.2	
" 10	0.4	2,500
" 14	0.7	2,930
" 15		2,750
" 17		2,920
" 19	1.0	
" 27		2,785
January 4		2,725
" 5	1.5	
" 7		2,850
" 16	2.0	3,010
" 25	3.0	3,060
February 2	4.50	3,000
" 11	6.00	3,060
" 22	8.00	2,950
March 6	10.00	3,010
" 16	Bled 20 c.c.; Killed March 19	2,950

This being such a large and definite multiple of a fatal dose, allowing for any possible hyper-resistance, the immunization was discontinued at this point and the animals killed about two weeks after the last inoculation. These excessively large amounts of *Rhus toxicodendron* were given in divided doses in different parts of the body.

In these three cases the total amount of the poison given was nearly 40 c.c. of the fluid extract. At the same time the four guinea-pigs were also treated by gradually increasing doses up to the point where they had received 2.5 and 3 c.c. of the poison (Table 6). In one

TABLE 6.

IMMUNIZATION OF FOUR GUINEA-PIGS.

(Weight of guinea-pigs varies from 460 to 950 grams.)

Date	Dosage (*Rhus tox.*)
	c.c.
January 16	0.1
" 19	0.2
" 23	0.3
" 28	0.5
February 4	0.75
" 11	1.00
" 21	1.35
March 2	1.80
" 11	2.50
" 19	Two smaller animals killed
" 20	3.00 given to other animals Killed April 3

of these a slight area of necrosis developed in the skin, while in the others the skin remained normal. After the animals were killed, no albumen or but a trace was found in the urine. From these experiments it may be safely concluded that in both rabbits and guinea-pigs an active immunity to *Rhus toxicodendron* may be established, the final amount of the poison administered being at least six or seven times a fatal dose. Beyond this point there is little object in pushing the immunization since such large volumes of the poisonous extract would be necessary as to damage the skin and subcutaneous tissues before neutralization. Furthermore we have given a sufficiently high multiple of a fatal dose as to indicate a definite artificial active immunity. We have therefore in *Rhus toxicodendron* a poisonous glucoside exerting a selective action upon the epithelial cells of the skin and kidney, by the gradual introduction of which immunity to fatal doses may be established.

NATURE OF *RHUS TOXICODENDRON* IMMUNITY.

Since we are working with a non-proteid substance it is necessary to inquire particularly into the nature of the immunity here described.

Are we dealing with an increased resistance on the part of these animals similar to that shown to morphine, arsenic, and to certain alkaloids like cocaine, or with a real immunity in which the body has produced substances actually uniting with and neutralizing the poison? This is an important question, since it has always been supposed that animals may become accustomed to a number of non-proteid poisons without producing any antitoxin for them. It was necessary therefore to test the blood serum of these immune animals, and to determine what capacity it had, if any, of neutralizing fatal doses of the poison for susceptible animals. For this study guinea-pigs were used exclusively. The great variation in susceptibility of rabbits, which I have already pointed out, would render experiments with them exceedingly difficult to interpret. Fortunately with guinea-pigs we can estimate the fatal dose within fairly narrow limits. Nevertheless we are confronted with a difficult problem, since it is impossible to mix the serum and the poison in varying proportions, as is done with ordinary antitoxins. It must be remembered that we have an alcoholic fluid extract of *Rhus toxicodendron* upon the addition of serum

to which a turbid precipitate is at once produced. It was determined to give the poison and the serum separately in different parts of the body, and I have preferred to employ the severer test of giving the poison first and the serum subsequently. The immune rabbits were bled at appropriate intervals, preferably 12 to 14 days after an injection, and the serum thus obtained was employed upon guinea-pigs to determine its neutralizing powers. In all cases we have found that the serum from these immune animals is antitoxic, and will neutralize definite multiples of fatal doses. Thus in the serum marked Rabbit No. 4, Table 7, it will be seen that 1 c.c. of this serum neutralizes 0.5 c.c., 0.75 c.c., 1 c.c. and 1.5 c.c. of the poison, while 2 c.c. of the

TABLE 7.

ANTITOXIC PROPERTIES OF SERUM FROM IMMUNE ANIMAL.

Serum from Rabbit 4, Bled after Dosage of 4.50 c.c. Tested on Guinea-Pigs.

Grams Weight	Dosage	Result
260	1 c.c. serum + 0.5 c.c. *R. tox.*	No effect
305	1 c.c. serum + 0.75 c.c. *R. tox.*	No effect
405	1 c.c. serum + 1 c.c. *R. tox.*	No effect
405	1 c.c. serum + 1.5 c.c. *R. tox.*	No effect
515	2 c.c. serum + 2 c.c. *R. tox.*	No effect

CONTROL.

Guinea-pig given 1 c.c. *R. tox.* found dead on 13th day.

The *Rhus toxicodendron* is given subcutaneously and the serum after the *Rhus toxicodendron*, but on the other side of the body.

serum will neutralize 2 c.c. of the *Rhus toxicodendron*. Therefore 1 c.c. of the serum will neutralize five or six times a fatal dose for the animal in question. These test animals were kept under observation for a period of six weeks. With the serum from another immune animal larger quantities of the *Rhus toxicodendron* were employed. As may be seen from Table 8, Rabbit No. 9, 2 c.c. of the serum neutralized 3 c.c. of the *Rhus toxicodendron*, but some necrosis developed at the point of inoculation. One c.c. of the serum neutralized 2.5 c.c. of the *Rhus toxicodendron*, however, with no slough at the site of injection, while with smaller quantities of the serum the neutralization was not accomplished. The same animal, Rabbit No. 9, was further immunized, a final dose of 10 c.c. being given. After this large dose its serum had an increased power of neutralizing the poison. With 1 c.c. and 0.5 c.c. quantities 2.5 c.c. of the poison was

TABLE 8.

ANTITOXIC PROPERTIES OF SERUM OF RABBIT NO. 9; BLED 11 DAYS AFTER A DOSAGE OF 7.50 C.C. *Rhus tox.*

(Tested Feb. 25 and March 2.)

Grams	Dosage	Result
600.....	2 c.c. serum+3 .c.c. *R. tox.*	Some necrosis at site of inoculation. Complete recovery
530.....	1 c.c. serum+2.5 c.c. *R. tox.*	No effect
400.....	0.5 c.c. serum+2.5 c.c. *R. tox.*	Death, 16 days
400.....	1 c.c. serum+1 c.c. *R. tox.*	No effect
420.....	1 c.c. serum+0.5 c.c. *R. tox.*	Death, but no lesions of *Rhus tox.* No slough and no nephritis
	CONTROLS.	
530.....	2 c.c. *Rhus tox.*	Death, 17 days
410.....	0.5 c.c. *Rhus tox.*	Death, 4 days
380.....	0.25 c.c. *Rhus tox.*	Recovery

neutralized to such an extent as to show no slough where the poison was injected, the animals dying, however, at a late date from nephritis. One c.c. again completely neutralized 1.5 c.c. of the poisonous extract and 0.25 c.c., a dose of 2 c.c.

It is thus seen that the serum of these animals immunized to *Rhus toxicodendron* contains substances neutralizing this poisonous glucoside when both are injected into susceptible animals, and the immunity is therefore an antitoxic immunity.

Sera from a number of other animals immunized to this poison have been tested and in all cases they have been found to be antitoxic in character. The tables already given are typical of the reactions of such sera and probably represent as strong a serum as small animals can be made to produce. Larger animals, such as goats, have already been immunized and the antitoxic strength of their serum will be tested as soon as opportunity is afforded. Should serum from large animals have a high degree of antitoxic power, the use of such serum would be justified in severe cases of poisoning by *Rhus toxicodendron*, and there is a reasonable possibility that it may acquire a definite place in practical therapy.